The Effects of Cyclopropane and Halothane on the Biosynthesis of Norepinephrine in Vivo:

Conversion of ^{14}C-Tyrosine to Catecholamines

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The rate of *in vivo* synthesis of norepinephrine (NE) in the heart and the brain was estimated in rats. ^{14}C-Tyrosine was infused intravenously at a constant rate for various time periods up to one hour. Measurement of plasma tyrosine and tissue NE and their specific activities allowed the calculation of fractional rate constant and rate of synthesis of NE, assuming an open single-compartment system in a steady state. Light anesthesia with 15 per cent cyclopropane or one per cent halothane in 25 per cent oxygen did not alter the NE level, fractional rate constant or rate of NE synthesis in the heart. Halothane significantly reduced the fractional rate constant of NE in the brain, but the rate of synthesis did not change. Cyclopropane and halothane appeared not to affect the enzyme systems concerned with NE synthesis.

WE HAVE REPORTED that cyclopropane and halothane do not appear to affect the uptake and release of norepinephrine (NE) by peripheral adrenergic nerves.[1] In the present study we examined the effect of anesthetics on the biosynthesis of NE *in vivo*.

In man cyclopropane elevated the plasma levels of NE.[2, 3] Li *et al.*[4] reported that in dogs cyclopropane increased the myocardial NE content. The increase was quite marked, ranging from 41 per cent in the right atrium to 110 per cent in the right ventricle after three hours of anesthesia. The source of NE for such an increase is of interest. One possibility is that NE, being released from the adrenal medulla, carried by blood, is taken up by the adrenergic nerves. The adrenal medulla could provide the NE if cyclopropane does indeed cause a release from it.

Another possibility is that cyclopropane could accelerate the synthesis of NE or interfere with its degradation. Intraneuronal degradation of NE depends upon monoamine oxidase (MAO). Studies of serotonin metabolism indicated that in rats MAO activity is not inhibited by anesthesia.[5] Catechol-O-methyltransferase (COMT) is believed to inactivate catecholamine (CA) extraneuronally. While COMT inhibition may prolong the action of released NE, it is not expected to increase the tissue NE levels[6] Therefore, it seems relevant to study the effects of anesthetics, particularly cyclopropane, on NE synthesis.

A method to measure the rate of NE synthesis *in vivo* has been developed. Following intravenous infusion of ^{14}C-tyrosine (the radioactive precursor of CA), plasma tyrosine and tissue NE levels and their specific activities were determined. From these the fractional rate constant and the synthesis rate of tissue NE can be calculated.[7]

Methods

Male Sprague-Dawley rats, each weighing about 200 g, were fasted for 16 hours before the experiment. The animals were placed in individual chambers as described in a previous paper.[1] Groups of four or more rats were anesthetized with 15 per cent cyclopropane or 1

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per cent halothane diluted with 25 per cent oxygen–75 per cent nitrogen. The righting reflexes were abolished. After one hour of anesthesia, ^{14}C-L-tyrosine (uniformly labelled, 365 mcuries/mM, New England Nuclear Corp.) was infused intravenously at a constant rate of 50 μcuries/hr per animal for various time periods up to one hour. Control experiments were done with animals breathing 25 per cent oxygen–75 per cent nitrogen during ^{14}C-tyrosine infusion.

At the end of the infusion the rats were decapitated. Blood was collected in tubes containing heparin. Brains and hearts were immediately removed, rinsed in water, blotted dry, and frozen for subsequent tyrosine and NE assay. The plasma was also frozen until analyzed for tyrosine.

Figure 1 outlines the procedures for the separation and assay of tyrosine and catecholamines. Details have been reported elsewhere. Tyrosine was assayed according to the method described by Udenfriend,[8] and NE according to that of Brodie *et al.*[9] Aliquots of appropriate samples were counted for radioactivity in Bray's mixture[13] with a liquid scintillation spectrometer (Packard Instrument Co.). Standards of ^{14}C-labelled tyrosine and NE were carried through the entire procedure. The recovery of tyrosine was about 95 per cent and that of NE, 60 per cent. Counting efficiency was determined by internal standard (^{14}C-toluene 25 λ, specific activity, 4.36×10^6 dpm/ml). The specific activities of NE were corrected for the loss of one carbon atom when the labelled tyrosine was converted to NE.

Portions of the Rexyn column eluate containing tyrosine were freeze-dried. The residue was restituted in 0.1 N hydrochloric-acid and the purity of tyrosine determined by paper

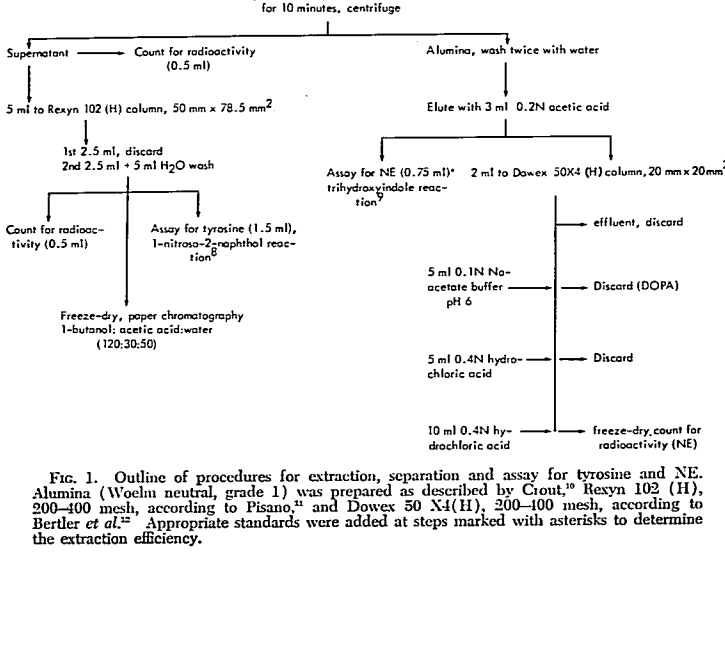

FIG. 1. Outline of procedures for extraction, separation and assay for tyrosine and NE. Alumina (Woelm neutral, grade 1) was prepared as described by Crout,[10] Rexyn 102 (H), 200–400 mesh, according to Pisano,[11] and Dowex 50 X4(H), 200–400 mesh, according to Bertler *et al.*[12] Appropriate standards were added at steps marked with asterisks to determine the extraction efficiency.

chromatography. Two radioactive compounds were found as described by Lewander and Jonsson,[14] tyrosine ($R_f = 0.45$) and an unknown material ($R_f = 0.21$). Specific activities of plasma and tissue tyrosine have been corrected for the presence of this contaminant.

Calculations for the fractional rate constant (k) and synthesis rate of NE are based on the assumption of an open single-compartment system. Derivation of equations for such a model system has been described.[7] Briefly, when ^{14}C-tyrosine is infused intravenously at a constant rate, the plasma tyrosine specific activity [T] would be expected to change with time as

$$\frac{d[T]}{dt} = A - k_T[T] \quad (1)$$

where A is the apparent rate of increase in tyrosine specific activity in the plasma compartment, k_T, the fractional rate constant of plasma tyrosine and t, duration of infusion. On integration and imposing the condition that $[T] = 0$ at time zero equation (1) becomes

$$[T] = \frac{A}{k_T}(1 - e^{-k_T t}) \quad (2)$$

With an open single-compartment system in a steady state where the amine levels presumably are maintained by equal rates of formation and efflux, the change in tissue radioactive NE(NE*) with time would be related to ^{14}C-tyrosine (T*):

$$\frac{dNE^*}{dt} = k_1 T^* - k_{NE} NE^* \quad (3)$$

where k_{NE} is the fractional rate constant of tissue NE and k_1, that of hydroxylation of tyrosine, the rate-limiting step in NE synthesis.[15] It is assumed that a rapid equilibration exists between plasma tyrosine and intraneuronal tyrosine.

During steady state $k_1 T$ must equal to $k_{NE}NE$ if the levels of the two intermediaries in the biosynthesis of NE (DOPA and dopamine) are negligible and their fractional rate constants are greater than k_1. Upon substitution and integration

$$[NE] = \frac{A}{k_T}\left\{1 + \frac{1}{k_{NE} - k_T}(k_T e^{-k_{NE}t} - k_{NE} e^{-k_T t})\right\} \quad (4)$$

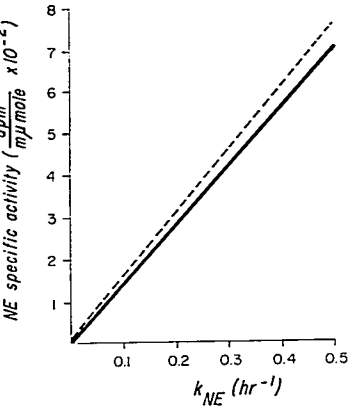

FIG. 2. Relationship between NE specific activity and fractional rate constant of NE (k_{NE}) after intravenous infusion of ^{14}C-tyrosine at 50 μc/hr for one hour. Solid line is for control experiments and dashed line, during cyclopropane or halothane anesthesia.

is obtained, where [NE] is the specific activity of NE, and equal to zero at time zero. [NE] is measured. The apparent rate of change in plasma tyrosine specific activity, A, and k_T are calculated from equation (2). Time of infusion, t, is known. From these k_{NE} can be derived. In practice a graph can be constructed to express the relationships between [NE] and k_{NE} for one hour of ^{14}C-tyrosine infusion (fig. 2).

Anesthesia was found to change the fractional rate constant of plasma tyrosine, k_T. Therefore, the relationship between [NE] and k_{NE} for anesthetized animals is slightly different from that of controls (fig. 2).

The rate of NE synthesis is obtained by multiplying the steady NE level with k_{NE}. For example, if the brain NE level is 0.44 μg/g and the k_{NE}, 0.25 hr^{-1}, the rate of NE synthesis is $0.44 \times 0.25 = 0.11$ μg/g/hr. The turnover time, the period required for the complete turnover of tissue NE store, would be NE level/synthesis rate, in this example, $0.44/0.11 = 4$ hours.

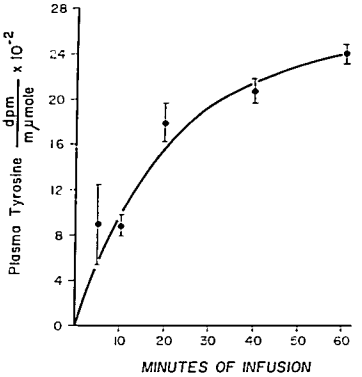

FIG. 3. Increase of the plasma tyrosine specific activity during ^{14}C-tyrosine infusion (50 μc/hr) in control animals. Each value is the mean ± SE from at least five animals. The curve represents the best-fit values according to equation (2).

Students' *t* test was used to assess the difference between control and experimental values.

Results

Intravenous infusion of ^{14}C-tyrosine at the rate of 50 μcuries/hr produced a curvilinear increase in plasma tyrosine specific activity, [T]. Figure 3 shows the results from control animals breathing 25 per cent oxygen during the infusion. Solution of equation (2) is

$$[T] = \frac{120 \text{ dpm/m}\mu\text{mole/min}}{0.047/\text{min}} (1 - e^{(0.047/\text{min})}),$$

where 120 dpm/mμmole/min is the apparent rate of increase of tyrosine specific activity in the plasma compartment, A, and 0.047/min equals the fractional rate constant of plasma tyrosine, k_T. In animals anesthetized with cyclopropane or halothane, solution of equation (2) from measured values of [T] at various times gave a k_T of 0.042/min. Accordingly, the average lifetime for a plasma tyrosine molecule is about 21 minutes in control animals and 24 minutes in anesthetized animals.

Halothane and cyclopropane anesthesia for up to two hours (one hour prior to and one hour during infusion) did not change the plasma and tissue levels of tyrosine. Specific activities of plasma and heart tyrosine were significantly higher during anesthesia, but in the brain the specific activities of tyrosine were similar to those in control animals (table 1).

Table 2 presents the mean NE level in the heart, its specific activity, fractional rate constant (k_{NE}), synthesis rate and turnover time in control and anesthetized animals. NE levels and specific activities were measured after one hour of ^{14}C-tyrosine infusion. The amine level increased slightly during cyclopropane anesthesia but the change was not statistically significant ($P > 0.05$). Cyclopropane and halothane did not affect k_{NE} or the rate of NE synthesis. It takes about 20 hours for the myocardial NE stores to turn over under control conditions and during anesthesia.

In the brain cyclopropane again did not appear to change the k_{NE} and the synthesis rate of NE significantly. The NE specific activity during halothane anesthesia was less than that of control. Accordingly, k_{NE} decreased. The

TABLE 1. Plasma and Tissue Tyrosine Levels and Specific Activities Following ^{14}C-Tyrosine Infusion*

	Plasma		Heart			Brain		
	Level (μg/ml)	Specific Activity (dpm/ mμmole)	Level (μg/ml)	Specific Activity (dpm/ mμmole)	Specific Activity Ratio Heart/ Plasma	Level (μg/ml)	Specific Activity (dpm/ mμmole)	Specific Activity Ratio Brain/ Plasma
Control (7)	11.8 ± 0.87	1,906 ± 181	19.0 ± 1.14	1,258 ± 83	0.62 ± 0.015	17.2 ± 1.07	1,030 ± 105	0.54 ± 0.043
Halothane (8)	11.1 ± 0.46	2,669 ± 182††	17.2 ± 0.66	1,686 ± 85††	0.64 ± 0.037	14.8 ± 0.54	1,188 ± 59	0.42 ± 0.031**
Cyclopropane (8)	11.2 ± 1.0	2,592 ± 200**	18.7 ± 0.96	1,601 ± 89†	0.59 ± 0.025	16.4 ± 1.17	1,223 ± 70	0.50 ± 0.01

* Values are means ± SE. ^{14}C-Tyrosine was infused intravenously at a rate of 50 μc/hr for one hour. Numbers of animals are indicated in parentheses.
** $P < 0.05$, † $P < 0.02$ and †† $P < 0.01$ when compared with controls.

TABLE 2. The Effects of Cyclopropane and Halothane on the Heart NE Level and Synthesis in Rats*

	NE Level (μg/g)	Specific Activity (dpm/mμmole)	k_{NE} (hr^{-1})	Synthesis Rate (μg/g/hr)	Turnover Time (hr)
Control (8)	1.11 ± 0.07	81 ± 8	0.052 ± 0.005	0.057 ± 0.005	21.15 ± 2.85
Halothane (6)	1.13 ± 0.06	83 ± 8	0.048 ± 0.005	0.053 ± 0.005	21.98 ± 2.00
Cyclopropane (7)	1.25 ± 0.08	83 ± 6	0.046 ± 0.003	0.057 ± 0.004	22.26 ± 1.70

* Values are mean ± SE, obtained after two hours of anesthesia and one hour of ^{14}C-tyrosine infusion (50 μc/hr).
Numbers of animals are indicated in parentheses.

TABLE 3. The Effects of Cyclopropane and Halothane on Brain NE Level and Synthesis in Rats*

	NE Level (μg/g)	Specific Activity (dpm/mμmole)	k_{NE} (hr^{-1})	Synthesis Rate (μg/g/hr)	Turnover Time (hr)
Control (7)	0.44 ± 0.017	379 ± 23	0.25 ± 0.014	0.11 ± 0.006	4.16 ± 0.30
Halothane (8)	0.51 ± 0.022**	312 ± 29	0.18 ± 0.017†	0.09 ± 0.006	5.82 ± 0.56**
Cyclopropane (8)	0.50 ± 0.03	368 ± 18	0.22 ± 0.01	0.11 ± 0.01	4.64 ± 0.24

* Values are mean ± S.E., obtained after two hours of anesthesia and one hour of ^{14}C-tyrosine infusion (50 μc/hr).
Number of animals in parentheses.
** $P < 0.05$ and † $P < 0.02$ when compared with controls.

NE level increased with borderline significance and the rate of synthesis (product of NE level and k_{NE}) was about the same as that of the control group (table 3).

Discussion

The calculation of NE synthesis from ^{14}C-tyrosine conversion assumes an open single-compartment system for NE formation and efflux in adrenergic nerves. Efflux includes release and degradation. According to this hypothetical model, if the tissue NE level does not vary during the period of observation, the amount of NE synthesized must equal the amount degraded or released. The model also assumes that the newly-formed NE is being handled in the same manner as that already present in the store, that is, uniformly distributed in a single pool. The validity of this hypothesis has been discussed in another publication.[7] Fractional rate constant and synthesis rate of NE in the brain and the heart estimated with this method closely approximate those obtained with other techniques. These include the use of α-methyl tyrosine or tracer doses of radioactive NE. By applying the principles of steady-state kinetics, the rate of NE synthesis has been measured from the rate of decline of NE levels after administering α-methyl tyrosine, a tyrosine hydroxylase inhibitor, or from the rate of decline of NE specific activity after injecting a tracer dose of radioactive NE.[9, 16]

The methods using radioactive NE or α-methyl tyrosine are not considered suitable for the present study. Both require large numbers of animals and long periods of observation, at least eight and preferably 24 hours, as the turnover time of heart NE is approximately 20 hours. It would be impractical to maintain a reasonably physiologic state under anesthesia for such a duration.

The utility of the method with ^{14}C-tyrosine has been tested with experimental conditions known to change the synthesis rate of NE. In rats chronic treatment with a MAO inhibitor (pargyline) elevates tissue NE levels. Through feedback inhibition of tyrosine hydroxylase, NE is synthesized at a slower rate.[17] Adrenal demedullation removes a source of catecholamines in the periphery and increases the rate of NE synthesis in the heart (fourfold) but not in the brain.[7] Presumably, catecholamines released from the adrenal medulla contribute to maintain the peripheral adrenergic homeostasis, but not that in the brain because of the blood–brain barrier.

The results from the present study show that

under the conditions of the experiment cyclopropane and halothane do not change the NE level and rate of synthesis in the rat heart. A few possibilities may be offered to explain these negative findings. The rat may not respond to cyclopropane or halothane in the same way as the dog or man. The anesthetic concentration (dose) used is not high enough to produce an effect on the adrenergic system, or perhaps the action of cyclopropane in increasing the plasma and tissue NE levels as reported [2, 3, 4] is not a primary drug effect.

The question of species difference can be resolved with similar experiments in another species, for example, dogs. However, there is no reason to suspect that the enzyme systems for NE synthesis and degradation are radically different among species. This is, of course, subject to further studies.

Only light anesthesia with a given anesthetic concentration was studied here. Considerations in providing adequate ventilation make it impractical to study deeper levels of anesthesia in a large group of animals. Furthermore, deep anesthesia may result in physiologic changes which, in turn, affect the system under study. Results reported in a previous paper [1] concerning the effect of cyclopropane on NE release illustrate this problem. In dogs cyclopropane did not change the pattern of NE release from the heart when the arterial pressure was not altered markedly. NE release increased when there was considerable hypotension. Increased NE release can be expected to increase the rate of synthesis. In any case, cyclopropane and halothane in concentration adequate for light anesthesia appear not to affect the rate of NE synthesis.

Li *et al.*[4] explained the increased NE levels in the dog heart on the basis of increased sympathetic activity. Increased sympathetic activity indeed accelerates NE synthesis, but it has not been reported to increase the tissue NE level. In the guinea pig vas deferens (*in vitro*) [18] and in the rat submaxillary gland (*in vivo*) [19, 20] sympathetic nerve stimulation increased the incorporation of radioactive tyrosine to NE. NE synthesis apparently is accelerated through the rate-limiting step of tyrosine hydroxylation.

Physiologic stresses also increase the rate of NE synthesis. Exposure to cold,[21, 22] exercise [22] or hypercapnia [23] (and Ngai, S. H., unpublished data) are shown to accelerate NE turnover in adrenergic tissues, presumably through sympathoadrenal activation. But, again, the tissue NE levels do not increase. Therefore the explanation offered by Li *et al.*[4] for the elevated myocardial NE levels seems untenable. The question of the source of increased myocardial and plasma NE levels during cyclopropane anesthesia is not answered by our experiments. A possible source is the adrenal medulla. The effect of anesthetics on the turnover of adrenal NE remains to be studied.

In respect to the broader question to explain the apparent lack of circulatory depression by cyclopropane, the action of this agent on the central regulatory mechanism is still controversial. In the periphery, cyclopropane does not appear to affect NE uptake or release.[1] Monoamine oxidase activity is not inhibited.[5] Inhibition of catechol-O-methyltransferase by cyclopropane can possibly potentiate the adrenergic transmitter action,[24] but this effect may be expected to play a minor role *in vivo*. *In vitro* the enzyme activity is inhibited to the extent of only 29 per cent in the presence of 100 per cent cyclopropane.[24] Results of the present study show that cyclopropane does not influence NE synthesis in the heart and the brain.

Cyclopropane-induced change in the receptor sensitivity thus would seem to be the only and most likely mechanism to explain the circulatory action of this anesthetic. Responses of the aortic strip of the rabbit [25] and the nictitating membrane of the cat [26] to catecholamines are exaggerated by cyclopropane. Davis *et al.* found that epinephrine increases the rate of diastolic depolarization (phase 4) in isolated canine Purkinje fibers and that cyclopropane enhances this response.[27] In anesthetized dogs the pressor effect of norepinephrine is potentiated about twofold by cyclopropane (Ngai, S. H., unpublished data). This action of cyclopropane has been observed with other "receptor" systems, in that it increases the skeletal muscle responses to indirect and direct stimulation.[28] The biochemical basis for this change in receptor sensitivity should be of some interest.

During halothane anesthesia k_{NE} in the brain was significantly lower than the control

value (table 3). This difference may be more apparent than real, because the rates of NE synthesis were approximately the same. The increase in the brain NE level was statistically significant ($P < 0.05$), but in terms of magnitude, it was about equal to that seen during cyclopropane anesthesia. Halothane may interfere with NE release from central adrenergic neurons—the turnover time is significantly longer; or, central depression during anesthesia may allow accumulation of NE. Available data preclude further speculation in this area.

The brain:plasma ratio of tyrosine specific activity was lower with halothane anesthesia (table 1). Since this amino acid is actively transported from plasma to brain, halothane could inhibit tyrosine transport. Further studies may be warranted to examine the possible effects of anesthetics on the transport of amino acids or other biological substrates. However, it should be pointed out that in the tissue tyrosine has many metabolic pathways in addition to NE synthesis: for example, protein synthesis. Only a small and unknown fraction of the total tissue tyrosine, the fraction concerned with NE synthesis, is present in the adrenergic neurons and nerve terminals. In any case, data reported in tables 2 and 3 suggest that anesthetics, as represented by cyclopropane and halothane, do not affect the enzyme systems involved in the synthesis of NE.

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Muscle

METHOXYFLURANE Muscular relaxation caused by methoxyflurane was tested by its effect on electromyographic reflexes. The tibial nerve, which carries afferent and efferent fibers, was stimulated. This produces a quick and direct muscular response via the neuromuscular synapse (potential A) and a reflex via the spinal cord (potential B). During general anesthesia with methoxyflurane, potential A was not influenced while potential B was decreased and finally disappeared. (*Droh, R., Sollberg, G., and Gottaid, A.: Electrophysiological Investigations of the Muscle-relaxing Effects of Methoxyflurane, Der Anaesthesist 17: 51 (Feb.) 1968.*)

MUSCLE PAIN Postoperative muscle pain occurred in 104 of 500 patients following the use of succinylcholine. Pain occurred more frequently in women than in men. The highest incidence occurred in patients between 14 and 40 years of age. There is no direct relation between the amount of succinylcholine used and the incidence of pain. Neostigmine, 0.5 mg three times a day, caused a significant decrease in muscle pain. No explanation is available for this effect. (*Brochert, K.: The Problem of Muscle Pain after Succinylcholine, Der Anaesthesist 17: 189 (June) 1968.*)